Supplemental Figure Legends

Figure S1 – related to figure 1. Analysis of antibody peptide binding *in vivo*.

(A) U2OS cells were transfected with a construct encoding the N-terminus of mitoNEET fused to GFP and incubated with mitotracker to stain mitochondria. Colocalization of green and red fluorescence indicates that the mito-fusion protein was effectively recruited to mitochondria. (B) U2OS cells were transfected with indicated antibodies along with four tandem copies of their peptide epitope (Htt for both antibodies) fused to mito-mCherry. Note that normal mitochondrial morphology is perturbed upon expression of the four tandem copies of the Htt peptide fused to mito-mCherry (compare with (A)). Scale bars, 10 µm.

Figure S2 – related to figure 2. Optimizing the GCN4 antibody-peptide pair.

(A) HEK293 cells were transfected with the indicated constructs and 24 hr after transfection, images were acquired using spinning disk confocal microscopy. Maximum intensity Z-projections are shown. All scale bars, 10 µm. (B) HEK293 cells were transfected with a sfGFP-linker-mCherry fusion protein and images were acquired on a spinning disk confocal microscope. GFP and mCherry fluorescence intensities for single cells were quantified and values were plotted after background subtraction.

Figure S3 – related to figure 3. Single molecule imaging using the SunTag.

(A) Representative images of U2OS cells expressing either scFv-GCN4-GFP alone or together with $SunTag_{24x}$ are show. Bottom panels are enlargements of boxed areas. (B-I) U2OS cells were transfected with indicated $SunTag_{24x}$ constructs and 24 hr later were imaged by time-lapse spinning disk microscopy. (B,C,D,F) Individual fluorescent foci were tracked using Trackmate (Fiji) and mean square displacement

plots were generated using Matlab, data from a representative cell is shown in each case. (E) Representative image of H2B-SunTag$_{24x}$-GFP is shown. (G-H) Speed (G) and run length (H) of K560-SunTag$_{24x}$–GFP were measured in at least 10 different cells. (I) Representative image of Camsap2-SunTag$_{24x}$–GFP is shown. Scale bars, 10 µm (A) or 5 µm (E,I).

Figure S4 – related to figure 5. dCas9-SunTag can recruit many copies of scFv-GCN4-GFP to a genomic locus.

HEK293 cells were transfected with dCas9-SunTag$_{24x}$, scFv-GCN4-GFP and indicated sgRNAs. 24 hr after transfection, cells were imaged by spinning disk confocal microscopy. Images in (A) are maximum intensity projections of Z-stacks. Intensities of individual telomere foci were measured in ImageJ and specific telomere fluorescence was calculated by subtraction of diffuse nuclear background. Vertical set of dots in (B) represents individual telomere intensities in a single cell. Scale bars, 5 µm. (C) Individual telomeres were tracked using Trackmate (Fiji) and mean square displacement plots were generated using Matlab, data from representative cells is shown.

Figure S5 – related to experimental procedures. Optimizing scFv-GCN4-GFP expression levels

U2OS cells were transiently transfected with both scFv-GCN4-GFP-NLS and SunTag$_{24x}$-CAAX. Images of 18 cells were acquired by spinning disk confocal microscopy. For each cell, the GFP intensity of the entire cell was measured, as well as the GFP intensity of 20 SunTag foci and 20 areas that did not contain any foci (background). (A) The average of GFP intensity of the SunTag foci is plotted against the total cellular GFP signal. The 5 cells with lowest total GFP signal are shown in green, and the 5 cells with the highest total GFP signal are red. The brightest cells have on average 9-fold higher GFP signal, but the SunTag foci

are only 2.4-fold brighter. (B) The average SunTag foci signal was divided by the average cytoplasmic background signal to generate a signal-to-background value. The signal-to-background was then plotted against the total GFP expression in the same cell. Note that the signal-to-background value is largely independent of the total GFP expression level, likely because the unbound scFv-GCN4-GFP is sequestered in the nucleus.

Supplemental Movie legends

Movie S1 Related to Figure 3. U2OS cells were transfected (from left to right) with $SunTag_{24x}$-CAAX-GFP, $SunTag_{24x}$-GFP, NLS-$SunTag_{24x}$-GFP and mito-mCherry-$SunTag_{24x}$-GFP. 24 hr after transfection cells were imaged using spinning disk confocal microscopy. The movies were acquired under continuous illumination for 20, 10, 10 and 2.5 sec. In the right movie, cells were also treated with mitotracker to stain mitochondria.

Movie S2 Related to Figure 3. U2OS cells were transfected H2B-$SunTag_{24x}$-GFP and 24 hr after transfection cells were imaged using spinning disk confocal microscopy. The movie was acquired under continuous illumination for 9 sec with 300 msec integration time per image (30 images total).

Movie S3 Related to Figure 3. U2OS cells were transfected K560-$SunTag_{24x}$-GFP and 24 hr after transfection cells were imaged using spinning disk confocal microscopy. The movie was acquired under continuous illumination for 30 sec with 200 msec integration time per image (150 images total).

Movie S4 Related to Figure 3. U2OS cells were transfected Camsap2-$SunTag_{24x}$-GFP and 24 hr after transfection cells were imaged using spinning disk confocal microscopy. The movie was acquired under continuous illumination for 25 sec with 500 msec integration time per image (50 images total).

Movie S5 Related to Figure 3. U2OS cells were transfected Kif18b-SunTag$_{24x}$-GFP and 24 hr after transfection cells were imaged using spinning disk confocal microscopy. The movie was acquired under continuous illumination for 10 sec with 200 msec integration time per image (50 images total).

Movie S6 Related to Figure 3. U2OS cells were transfected K560rig-SunTag$_{24x}$-GFP and mCherry-α-tubulin and 24 hr after transfection cells were imaged using spinning disk confocal microscopy. The movie was acquired under continuous illumination for 60 sec with 600 msec integration time per image (100 images total). Movie on the far right shows a cropped region of the cell in which a single microtubule is manually tracked that undergoes translocation (arrows).

Movie S7 Related to Figure 4. U2OS cells were transfected K560-SunTag$_{24x_v4}$-GFP and 24hr after transfection cells were imaged using spinning disk confocal microscopy. The movie was acquired under continuous illumination for 50 sec with 100 msec integration time per image (500 images total).

Supplemental table 1. Plasmid sequences.

Names and sequences of all plasmids used in this study

Supplemental Experimental Procedures

Optimizing SunTag expression levels

To obtain the optimal ratio between scFv-GCN4-GFP and the peptide scaffold for single molecule imaging experiments, the scFv-GCN4-GFP was expressed under control of different promoters and the

best expression level was empirically determine for each SunTag fusion protein. While higher expression level of the scFv-GCN4-GFP resulted in brighter SunTag foci, this effect was not linear; a 9-fold increase in expression levels of scFv-GCN4-GFP resulted in only a 2.4-fold increase in SunTag foci brightness (Figure S5A). However, since the signal-to-background remained largely constant over a wide range of scFv-GCN4-GFP expression levels (Figure S5B), likely due to the buffering effect of the NLS on the svFv-GCN4-GFP (which sequesters unbound antibody to the nucleus), generally higher expression of scFv-GCN4-GFP results in better imaging conditions.

Transfection, infection and generation of cell lines.

To generate lentivirus, HEK293 cells were plated in 6-well plates, and 24 hr after plating, cells were transfected with lentiviral packaging plasmids. 24 hr after transfection, the cell culture medium was replaced, and 72 hr after transfection the cell medium containing lentiviral particles was harvested and either used directly to infect cells or frozen at -80°C for later use. To generate K562 cells stably expressing dCas9-SunTag$_{10x_v4}$ and scFv-GCN4-GFP-NLS-VP64, cells were infected with freshly harvested lentivirus diluted 1:3 in RPMI cell culture medium and incubated for 24 hr in virus-containing medium. Our initial experiments with the polyclonal K562 cell line expressing dCas9-SunTag$_{10x_v4}$ and scFv-GCN4-GFP-NLS-VP64 generated in this way revealed that only ~40% of cells showed robust transcriptional activation, possibly due to cell-to-cell variation in transgene expression level. We therefore plated the K562 cells expressing dCas9-SunTag$_{10x_v4}$ and scFv-GCN4-GFP-VP64 at one cell per well in a 96-well plate and isolated several monoclonal cell lines that showed uniform transcriptional activation. One clone (E3) was selected for further experiments. For all experiments involving transcriptional activation, K562 cells expressing dCas9-SunTag$_{10x_v4}$ and scFv-GCN4-GFP-VP64 were infected with lentivirus encoding a gene-specific sgRNA together with a puromycin resistance gene and either BFP or mCherry at an multiplicity

of infection (MOI) of less than one, so most cells received a single lentivirus. Cells were then treated with 1 µg/ml puromycin, where indicated, for 3 days to select for cells that expressed a sgRNA.

Microscopy

For time-lapse microscopy cells were grown in DMEM:F12 medium without phenol red, supplemented with 20 mM HEPES to maintain correct pH in the absence of added CO_2 and were imaged in a thermally-controlled chamber heated to 37°C. For single molecule imaging of the SunTag, 2x2 pixel binning was applied, resulting in a pixel size of 166 nm. For photobleaching experiments, a single point was illuminated for 500 msec using a dedicated 488 nm photobleaching laser which was run at 5 mW. Image acquisition before and after photobleaching was performed using spinning disk confocal microscopy as described above. Fluorescence intensities of GFP before and after photobleaching were determined for each time point and corrected for cellular background fluorescence signal.

sgRNA and primer sequences

sgRNA sequences used in this study are: Control TTCTCTTGCTGAAAGCTCGA, CXCR4 #1 GCCTCTGGGAGGTCCTGTCCGGCTC, CXCR4 #2 GCGGGTGGTCGGTAGTGAGTC CXCR4 #3 GCAGACGCGAGGAAGGAGGGCGC, CDKN1B #1 AAGGTCGCCGGCAGCTCGCT, CDKN1B #2 GAAGCCGGGACCTGGACCAG, CDKN1B #3 CTGCGTTGGCGGGTTCGCCG, CDKN1B #4 GGGCCCGGCGCTGCGTTGG. CDKN1B specific primers for qPCR: Fw GAGTGGCAAGAGGTGGAGAA and Rev GCGTGTCCTCAGAGTTAGCC.

SunTag nomenclature

Example: K560-SunTag$_{24x_v4}$-GFP

“K560” = Name of protein fused to peptide array.

“SunTag” = GCN4 peptides are fused to the K560 protein.

$_{24x}$ = 24 copies of the GCN4 peptides were used.

$_{\_V4}$ = The v4 version of the peptides was used

GFP = The K560 protein with fused peptides was co-expressed with the scFv-GCN4 antibody which was fused to sfGFP.

Plasmids published previously:

EB3-tdTomato (Wozniak et al., 2009)

sgTelomere and dCas9-GFP (Chen et al., 2013)